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Italian Society of Uro-Oncology

category and toxicities, but, of note, 56% of the high-risk patients developed GU/GI toxicity, while only the 25% of the low-risk ones did (odds ratio=3.75, *p*-value=0.16). *Conclusion:* This study may serve as a starting point for finding safe bladder constraints for salvage SBRT. On the other hand, no real constraints for the rectum were found but on the basis of the very few rectal/intestinal major toxicity events registered, one can assume the median values of the dose-volume points as a safe rectal dose–volume. Finally, patients' comorbidities need to be taken into careful consideration in patient selection for and planning of salvage SBRT.

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TRANSGLUTAMINASE-2 INHIBITION RESTORES P53 EXPRESSION PREVENTING ITS DEGRADATION BY AUTOPHAGIC PATHWAY IN ccRCC CELLS

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Background: Renal cell carcinoma (RCC) is mainly clustered into three heterogeneous groups of tumors, namely clear-cell renal cell carcinoma (ccRCC), the most common; papillary, and chromophobe (1). About one-third of patients with RCC will develop disease recurrence or distance metastases. Despite significant therapeutic improvements, the 5-year survival rate of patients with metastatic renal carcinoma remains poor (1). Many genes are involved in renal cancer, including *VHL, BAP1, PBRM1, SETD2, KDM5C, KDM6A, mTOR, PTEN, PIK3CA,* and *TP53* (1). In particular, it is emerging that the tumor suppressor TP53 seems to be involved in the progression of renal cancer. We recently described that TP53 protein is removed and inactivated by the autophagic system in ccRCC cell lines (2). Accordingly, the inhibition of autophagy leads to restoration of TP53 expression inducing the reduction of both cell proliferation and migration (2). TP53 protein may be 'caught' by the autophagic process through the enzyme transglutaminase-2 (TG2) (3). TG2 is a multifunctional enzyme that mainly catalyzes cross-linking and GTPase/ATPase reactions. This protein is involved in cell adhesion, migration, invasion, proliferation and epithelial to mesenchymal transition in different cancer types (3). Autophagy might be a route used by kidney cancer cells to degrade TP53 by cross-linking activity of TG2, thereby promoting cancer progression. Here, we studied the involvement of TG2 in autophagy-mediated TP53 degradation in different ccRCC cell lines. In particular, we analyzed the impact of TG2 inhibition on TP53 expression and the downstream effects in our ccRCC cellular models. *Materials and Methods:* Analysis of autophagy was carried out on paraffin-embedded ccRCC primary tissues and corresponding metastases by immunohistochemistry using specific antibodies recognizing the autophagic marker light chain 3 protein (LC3). Images were acquired using a microscope equipped with a CCD camera at 10× magnification and processed by ImageJ software. TG2 inhibition was performed in Caki-1, Caki-2 and KJ29 ccRCC cell lines as well as in HEK293 kidney control cells using the specific inhibitor (*S*)-benzyl(1-(4-(1-naphthoyl)piperazin-1-yl)-6-acrylamido-1-ox-ohexan-2-yl)carbamate (AA9). The levels of TG2, TP53 and beta-actin proteins were evaluated by western blotting in cells treated with and without AA9 (9 μM) for 24 h. Cell proliferation was analyzed by direct cell counting. Briefly, kidney cancer and control cells were seeded at the density of 25,000 per well in a 24-well plate in Dulbecco's modified Eagle's medium/F12 with 1% fetal bovine serum medium (control) and in the same medium containing TG2 inhibitor AA9 (9 μM). Cells were then cultured for 48 h and, after trypan blue staining, were directly counted by using a Burker chamber. Statistical analysis was performed by *t*-test; $p<0.05$ was considered statistically significant. *Results:* We found that the metastatic tissue was more autophagic than the matched primary tumor (Figure 1A), suggesting that autophagy is increased in advanced kidney carcinoma. We observed that the expression of TG2 was higher in kidney cancer cells than controls (Figure 1B); therefore, this enzyme could be involved in the fate of this cancer. The inhibition of TG2 treating different tumor cell lines with AA9 enhanced the expression of TP53 and reduced cell proliferation compared with untreated cells (Figure 1C and D). We also observed that the inhibition of TG2 by AA9 did not affect enzyme stability because the levels of TG2 after cell treatment with this compound remained unchanged (Figure 1B). *Discussion*: The role of autophagy in cancer is ambiguous because it has been reported that this pathway can act as a tumor-suppressive process eliminating carcinogenic elements or triggering a cell death mechanism in support of apoptosis. Nevertheless, in advanced tumors, cancer cells can take advantage of this recycling system providing energy to the cell when oxygen and nutrients are scarce. Thus, autophagy can enhance cell survival and promote tumor progression, suggesting a dual role in carcinogenesis for this biological process. Our results confirm that in advanced renal tumors, autophagy is increased; in fact,

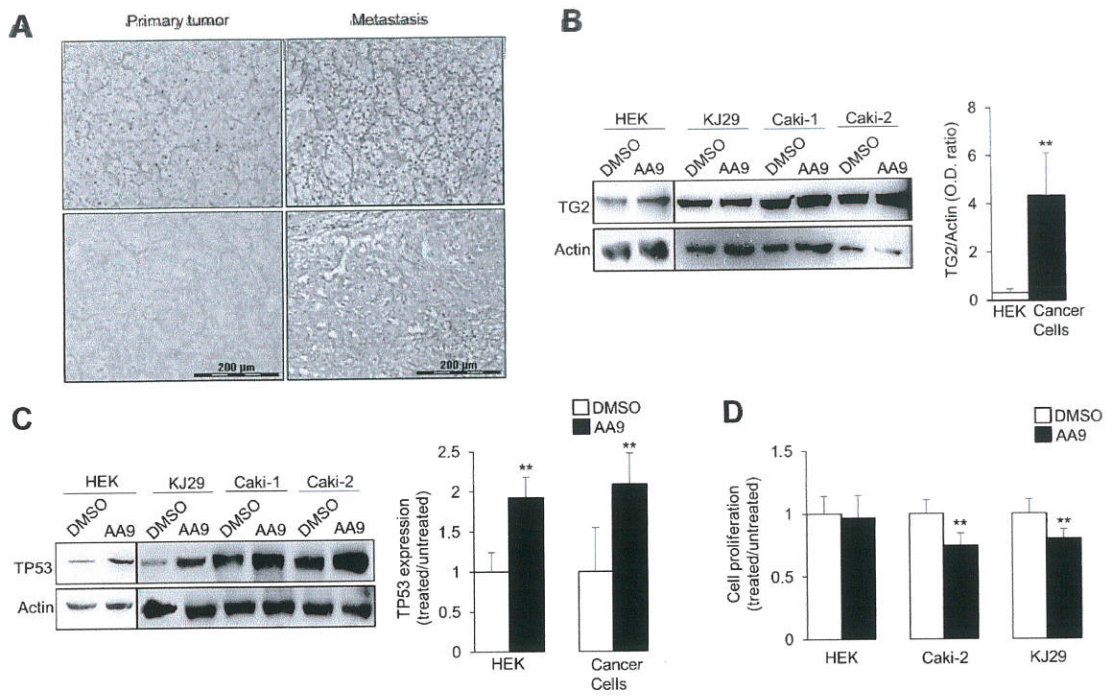

Figure 1. *Analysis of autophagy in kidney cancer tissues and evaluation of transglutaminase-2 (TG2) and TP53 expression in control and kidney cancer cells treated with AA9. A: Immunohistochemistry performed on primary tumor kidney tissues and corresponding metastases using antibodies recognizing the autophagic marker light chain 3 protein (LC3) showed greater staining in metastatic tissues than in primary tumors. B: Western blot analysis using an antibody to TG2 showed that kidney cancer cells (Caki-1, Caki-2 and KJ29) express higher levels of TG2 than HEK control cells (**p<0.01). C: The inhibition of TG2 by treatment with TG2 inhibitor AA9 did not change the expression of TG2 but increased the levels of TP53 compared with cells cultured in the presence of DMSO (vehicle). Data are expressed as the ratio of relative TP53 expression between treated and untreated cells (**p<0.01). D: The analysis of cell proliferation was performed in control and tumor cells (Caki-2 and KJ29) cultured in the presence of DMSO or AA9. Inhibition of TG2 significantly reduced cell proliferation in tumor cells but not in control cells. Cell proliferation was calculated as the ratio between cells treated with AA9 and untreated cells (**p<0.01). Data, expressed as the mean±standard deviation, were obtained from at least two independent experiments.*

distance metastases exhibited higher levels of autophagy than the corresponding primary tumors indicating that autophagy might be associated with tumor progression. As previously reported, the activation of autophagy contributes to TP53 degradation, trapping it in autophagosomes in kidney cancer cells through a mechanism mediated by TG2 (2, 3). The inhibition of TG2 using AA9 restored TP53 protein levels and reduced cell proliferation, confirming that TG2 may promote the degradation of TP53 by autophagy in kidney cancer. *Conclusion:* Our observations indicate that TG2 might represent a new therapeutic target for kidney carcinoma.

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RESIDENTS' LEARNING CURVE AFTER MORE THAN 1,000 PROSTATE MRI/TRUS TARGETED FUSION BIOPSIES

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